Life Science Applications

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Contents

› DLS (Size) Applications
› ELS (Zeta Potential) Applications
› SLS (Molecular Weight) Applications
› Flow Mode (Combining DLS and SEC) Applications
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Cumulants Analysis method

› The cumulants analysis is defined in the International Standard on Dynamic Light Scattering ISO13321 (1996)
  - Intensity weighted mean particle size (Z-average)
  - estimate of the width of the distribution (polydispersity index [pdi])

› Required:
  - dispersant refractive index
  - dispersant viscosity
Polydispersity Index (PdI)

\[
\%\text{Polydispersity} = \sqrt{\text{PdI}} \times 100
\]

DLS and Protein Stability

DLS is inherently sensitive to the presence of a few large molecules in a sample of predominantly small molecules and is an excellent tool to look at the early onset of aggregation and can be used to monitor:

- the effects of different additives in formulations
- protein thermal stability
- the effect of storage conditions
- changes due to pH or ionic concentrations
- conjugation of PEGs
- stability of nanoparticles (polymer or lipid based, coated or filled with protein or DNA)
- etc...
Stability Study of Recombinant Human Serum Albumins

- Albucull™
- Recombumin™
  - Preventing aggregation, oxidation, and surface adsorption of protein

- Aspects investigated:
  - Size:
    - dependence on concentration,
    - sample stability (time & storage conditions) - presence of aggregates
  - Thermal stability
  - pH dependence

- Measurements were done on Zetasizer Nano and Zetasizer APS
- Samples provided by Novozymes Biopharma UK Ltd

Light Scattering is Sensitive to Size

- Scattered light intensity $\alpha$ radius$^6$
Comparison Albucult™ and Recombumin™

Stock solutions
- Albucult (10%) in
  - 145mM NaCl
  - 8mM Sodium Octanoate
- Recombumin (20%) in
  - 145mM NaCl
  - 32mM Sodium Octanoate
  - 0.015mg/ml Polysorbate 80

No change in samples over time i.e. no aggregation

Electrostatic/Virial Effect at High Protein Concentration
- Addition of NaCl to suppress inter-protein repulsion and determine the size of the protein
- Hydrodynamic radius is 3.7+/−0.1nm for Recombumin and 3.6+/−0.12nm for Albucult
Thermal Stability of Albucult™ and Recombumin™

› Measurements settings
  ▪ Trend between 25°C and 85°C
  ▪ 1°C steps
  ▪ 20 seconds equilibration time at each step
  ▪ 1 measurement at each temperature
  ▪ Using Zetasizer APS

Albucult™ Thermal Trends

Intensity increases with concentration as expected
No visible concentration dependence within this range
The rate of change in scattering upon aggregation depends on the initial sample concentration

Intensity increases with concentration as expected
No visible concentration dependence within this range
The rate of change in scattering upon aggregation depends on the initial sample concentration
Protein Peak Size During Thermal Trend

Protein peak disappears at the same temperature for all concentrations.

Starting size different due to protein concentration effects as seen on earlier slides.

Variation in size largest for 0.5mg/ml as the signal-to-noise is low.

pH Stressed Recombumin™

- Mean hydrodynamic size of protein after 6 months, sample stored at 4°C
- Monomer ~3.54nm
- Dimer ~4.8nm
DLS Results Show Similar Trend as GP.HPLC Results

Percentage dimer for T=6month stability samples, 5°C, as determined by GP.HPLC

Results were kindly provided by Karl Nicholls, Novozymes Biopharma UK Ltd

Thermal Trends of pH Stressed Samples

- Measurements settings
- Trend between 25°C and 85°C
- 1°C increments
- 20 seconds equilibration time at each step
- 1 measurement at each temperature

- pH 3 least stable followed by pH 4
- pH 5, 6, 7, 9 and 10 show similar thermal stability
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Measuring High Conductivity Samples

› Capillary cell maximum conductivity is 200mS/cm (equivalent to approximately 2M NaCl)
› As conductivity increases, the measurements become more difficult
  • Joule heating
  • Electrode polarisation
  • Electrode degradation (blackening)
  • Sample degradation (e.g. aggregation)
› This is particularly problematic when working with proteins
Diffusion Barrier Method

› The automatic settings of the instrument try to minimize these potential problems as much as possible
› The use of the **diffusion barrier technique** will further help in minimizing any effects of the application of the field on the zeta potential results obtained

Diffusion Barrier Method

› The diffusion barrier technique protects the sample from degradation by keeping a sample plug (between 20 and 100µl) in a larger volume of the same dispersant

› Gel electrophoresis loading tips can be used to introduce the sample into the bottom of a folded capillary cell
The iso-electric point for the recHSA as determined here is around pH 4.6
IEF has given pI 5.1

Zeta potential measurements of rHSA

Contents
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ELS (Zeta Potential) Applications
**SLS (Molecular Weight) Applications**
Flow Mode (Combining DLS and SEC) Applications
Static Light Scattering

Static light scattering measures the time-averaged intensity of scattered light, from which the weight-averaged molecular weight ($M_w$) and 2nd virial coefficient ($A_2$) can be determined.

Debye plot: Lysozyme in PBS

Debye plot for Lysozyme in PBS showing:
- 1/Intercept = 14.6KDa
- Gradient = -3.23 x 10^{-4}
Using $A_2$ As A Stability Predictor

$A_2$ is closely correlated with sample solubility.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Molecular Weight (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme in 0.1M NaCl pH 7</td>
<td>15</td>
</tr>
<tr>
<td>Lysozyme in 4% NaCl pH 4.2</td>
<td>14.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>$A_2$ (ml mol/g)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme in 0.1M NaCl pH 7</td>
<td>0.000940</td>
</tr>
<tr>
<td>Lysozyme in 4% NaCl pH 4.2</td>
<td>0.000292</td>
</tr>
</tbody>
</table>

Measuring Mw and $A_2$ by static light scattering.
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Zetasizer µV Batch and Flow Mode Measurements

› Dynamic light scattering to measure size and estimated molecular weight
› Seconds to switch between batch and flow-modes
  ▪ Simply switch the cell
Zetasizer µV Batch and Flow Mode Measurements

> Combined solution for batch DLS and SEC-LS

- Batch measurements of size by DLS at 90º
  - Size
  - Thermal trends

- Add LS to an existing SEC system with:
  - 1 concentration detector for molecular weight
  - 2 concentration detectors for conjugate analysis

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Many thanks for your attention

Any questions?